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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 09/914,765 | 11/08/2001 | Preben Christensen | 60123.000002 | 4417 |

21967 7590 10/29/2003

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| EXAMINER | |
|---|---|
| SAUCIER, SANDRA E | |
| **ART UNIT** | **PAPER NUMBER** |
| 1651 | |

DATE MAILED: 10/29/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/914,765 | CHRISTENSEN ET AL. | |
| | Examiner | Art Unit | |
| | Sandra Saucier | 1651 | |

*-- Th MAILING DATE of this communication appears on the cov r sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *13 August 2003* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1,3-11 and 14-48* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-11 and 14-48* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *05 September 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. §§ 119 and 120**

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the international Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *8/13/03* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

## DETAILED ACTION

Claims 1, 3-11, 14-48 are pending and are considered on the merits.

### *Information Disclosure Statement*

The information disclosure statement filed 8/13/03 fails to comply with 37 CFR 1.98(a)(2), which requires a legible copy of each U.S. and foreign patent; each publication or that portion which caused it to be listed; and all other information or that portion which caused it to be listed. It has been placed in the application file, but the information referred to therein has not been considered.

Applicants' file has been converted into a image file. Either the cited references were not submited with the PTO 1449 or they were not scanned into the virtual file. A message has been sent to the scanning department, but it is possible that the references have been permanently misplaced. If you wish the cited reference to be considered, they should be resubmited for scanning.

### *Claim Rejections - 35 USC § 112*

NEW MATTER

Claims 47 and 48 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Insertion of the limitations where the samples are **simultaneously** analyzed with both a green and blue laser or **simultaneously** analyzed by two cytometers has no support in the as-filed specification. The insertion of this limitation is a new concept because it neither has literal support in the as-filed specification by way of generic disclosure, nor are there specific examples of the **simultaneous** analysis of samples with two lasers or two cytometers which would show possession of the concept of this use. The exemplified uses of two flow cytometers or two lasers are not performed simultaneously. This is a

matter of written description, not a question of what one of skill in the art would or would not have known. The material within the four corners of the as-filed specification must lead to the generic concept. If it does not, the material is new matter. Please carefully review the specification to determine the disclosed invention.

INDEFINITE

Claims 1, 3-11, 14-48 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

It is unclear what is intended by the recitation of "in the same determination routine" in claim 1, especially since a second determination can be made with a "subsample".

It is unclear what period of time is encompassed by the use of the phrase "substantially simultaneously" in claim 2. Does one second, minute, hour, day or week, etc. fall within or without the boundaries of the claim? The lack of a definition in the specification makes this limitation ambiguous

*Response to Arguments*

Applicants argue that the above phrases are defined on page 6, lines 11-13, and page 16, lines 29-33. A careful examination of the cited pages and lines was made; however, no references were seen to the above phrases. Thus, the rejection has not been overcome.

Claim 46 cannot be understood because the flow of the steps does not make sense. No formation of the recited subsamples is found in the claim. Are two or more separate diluted samples analyzed at the same time? The method as claimed cannot be interpreted.

*Claim Rejections - 35 USC § 102*

Claims 1, 3-6, 9, 16-19, 31, 32, 33, 36, 39, 41, 43, 45 remain/are rejected under 35 U.S.C. 102(b) as being clearly anticipated by GB 2 214 518 [J].

The claims are directed to a method of determining sperm concentration and % viability by selective staining in the same sample or subsample at substantially the same time.

GB 2 214 518 discloses a method of determining sperm concentration and % viability in the same sample in the same determinative routine at substantially the same time. See Example 1, where a sample is stained with PI and the fluorescence measured (F1), the sample is then permeabilized and the intensity (F2) is measured. A subsample is measured (F5) and the % viability is calculated. F2 is also proportional to the cell count and F2-F3 is correlated with cell concentration which is measured in the course of the process, see example. The determinations are considered to be made on the same sample or subsample and the determinations are considered to be performed "substantially simultaneously" or in the same determinative routine, as the routine is a FAC scan. Buffer is used to dilute the sperm, stabilization of pH sustains viability. The purpose in this method is the qualification of sperm for use in artificial insemination, where the biological utility of the sperm could be predicted in practice. (p.1, l. 7-14).

***Response to Arguments***

Applicants argue that GB 2 214 518 teaches the use of six samples prepared in different ways. However, careful reading of the example of '518 shows that the same sperm sample is used for the determination of live and dead sperm. The other "samples" are blanks. Blanks or controls or background samples are always used to zero readings in any type of mechanical quantitation presumably also in a cell cytometer. Thus, the sperm are first exposed to PI, quantitated, then exposed to a permeabilizing agent and quantitated. This is the use of the same sperm sample to quantitate both live and dead sperm substantially simultaneously as the determinations are

performed within seconds of each other. The same routine is used, that is the routine of measuring fluorescence. Thus, the method AS PRESENTLY CLAIMED is anticipated.

*Claim Rejections - 35 USC § 103*

Claims 1-11, 13-29, 31-33, 36-39, 41, 43, 44, 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over US 4,559,309 [B] or JP 8-332098 [L] or Live/Dead Sperm Viability Kit [U1] or Garner *et al.* [V1] or GB 2 214 518 [J] in combination with EP 586 183 [I] or WO 93/16385 [H].

The claims have been discussed above.

The references are relied upon as explained below.

US 4,559,309 teaches the determination of the proportion of live sperm to dead or dying sperm in a sample by staining with rhodamine 123/ethidium bromide and using flow cytometry to determine viability (col. 2, l. 66). The method may be used on fresh or frozen samples (col. 2, l. 16). The method allows the differential determination of dying cells as well as live and dead cells (col. 4, l. 5-21). The diluent contains FCS.

JP 8-332098 disclose a method of determining the live/dead proportion of a sperm sample by staining with two distinct fluorescent dyes and using flow cytometry.

Live/Dead Sperm Viability Kit discloses a method of determining the proportion of live sperm cells in a sample by staining with SYBR 14 and propidium iodide and analyzed by flow cytometry. The diluent contain BSA.

Garner *et al.* teach a method of viability determination of sperm using SYBR-14 and PI.

WO 93/16385 discloses a method of determining the total number of cells per unit volume of cell sample in a flow cytometer. A known number of particles having a known light scatter signal are added to a known volume of sample prior to analysis (abstract).

EP 586 183 discloses a method of determining absolute cell counts in a sample using a known number of fluorescent particles added to a known volume of sample, and using a flow cytometer (Summary of the Invention).

The addition of the method of WO 93/16385 or EP 586 183 to the method of US 4,559,309 or JP 8-332098 or Live/Dead Sperm Viability Kit or Garner *et al.* or GB 2 214 518 would have been obvious because both '385 or '183 suggest and generically teach the addition of microparticles of known quantity to a known volume of cells in order to be able to calculate the absolute number of cells in a sample analyzed by flow cytometry.

With regard to the use of a 25-75nM concentration of fluorochrome stain, Live/Dead Sperm Viability Kit states in the staining protocol that the examples are provided to guide researchers in the development of their own staining protocol, and that concentrations of reagents required for optimal staining may vary depending on density and other materials in the sperm sample. 100nM SYBR 14, used in the example, is the highest concentration recommended. In the absence of evidence to the contrary, given the guidance in the prior art, one of skill in the art may optimize the concentration required.

With regard to the number of control particles used per number of cells in the sample, EP 0 586 183 suggests using particles in a range of 0 to near the concentration of cells in the sample (page 5, l. 35). This is considered to be an optimization step which is well within the purview of one of skill in the art in the absence of evidence of unexpected results.

Claim 30 is rejected under 35 U.S.C. 103(a) as being unpatentable over US 4,559,309 [B] or JP 8-332098 [L] or Live/Dead Sperm Viability Kit [U] or

Garner *et al.* [V] or GB 2 214 518 [J] in combination with EP 586 183 [I] or WO 93/16385 [H] as applied to claims 1-11, 13-29, 31-33, 36, 37-39, 41, 43 and 44 above, and further in view of Clay *et al.* [W1].

The claim is further directed to the addition of PVA to the dilution medium.

Clay *et al.* teach that decrease in sperm motility due to dilution may be reduced by the addition of PVA or BSA, abstract.

The addition of PVA or the substitution of PVA for BSA in the dilution medium in the methods of US 4,559,309 or JP 8-332098 or Live/Dead Sperm Viability Kit or Garner *et al.* or GB 2 214 518 would have been obvious when taken with Clay *et al.* who teach the advantages of such an addition.

Claims 32-34, 39, 41, 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over US 4,559,309 [B] or JP 8-332098 [L] or Live/Dead Sperm Viability Kit [U1] or Garner *et al.* [V1] or GB 2 214 518 [J] in combination with EP 586 183 [I] or WO 93/16385 [H] as applied to claims 1-11, 13-29, 31, 36-38, 43 and 44 above, and further in view of Sexton [X1] or Januskauskas *et al.*[U2] or Belorkar *et al.* [V2] or Bostofte *et al.* [W2]

The claims are further directed to the use of the determination of the concentration and proportion of viable sperm in the sample to predict fertility and as a basis to adjust AI dosage.

Sexton teach that concentration and viability of sperm in an insemination dosage are directly correlated to fertility for turkey semen. Sexton also teach the use of determination of the concentration of viable sperm to adjust the insemination dosage in order to obtain high fertility rates.

Januskauskas *et al.* teach that membrane integrity (viability) and concentration of sperm from a cryopreserved sample is directly correlated with fertility in bulls.

Belorkar *et al.* teach that sperm concentration and viable sperm % are directly correlated with fertility in bulls in fresh ejaculates.

Bostofte *et al.* teach that semen quality is a function of sperm count (concentration) and viability and that semen quality is directly correlated with fertility in humans.

The prediction of fertility based on a determination of sperm concentration and viability which is determined by prior art methods as demonstrated above would have been obvious because many in the art have shown a direct correlation between sperm concentration and sperm viability with fertility.

Claims 35 and 40 are rejected under 35 U.S.C. 103(a) as being unpatentable over US 4,559,309 [B] or JP 8-332098 [L] or Live/Dead Sperm Viability Kit [U1] or Garner [V1] or GB 2 214 518 [J] in combination with EP 586 183 [I] or WO 93/16385 [H] as applied to claims 1-11, 13-29, 31-33, 36, 37-39, 41, 43 and 44 above, and further in view of Juonala *et al.* [X2] and/or Viudes-De-Castro *et al.*[U3].

Juonala *et al.* disclose that sperm viability is directly correlated with fertility which is measured by non-return rates and litter size.

Viudes-De Castro *et al.* disclose that sperm concentration has a direct correlation with fertility and litter size up to a threshold value.

The prediction of litter size which is correlated with fertility and predicted in the prior art by determination of sperm viability and sperm concentration would have been obvious because Juonala *et al.* have shown a direct correlation

between sperm viability with fertility, of which litter size is an element and Viudes-De-Castro *et al.* have demonstrated a direct correlation of litter size with sperm concentration up to a threshold value.

One of ordinary skill in the art would have been motivated at the time of invention to make this addition of microparticles to the sperm sample in order to obtain the results as suggested by the references with a reasonable expectation of success. The claimed subject matter fails to patentably distinguish over the state of the art as represented by the cited references. Therefore, the claims are properly rejected under 35 U.S.C. § 103.

In short, the staining method, counting method with an addition of an internal standard and use to which the results, namely sperm concentration and viability, are employed are all known in the art and the combination of these methods is not unobvious especially because it is known that fertility is correlated with absolute sperm count as well as with viability of the sperm. Attention to the exemplified method and presentation of unexpected results might advance prosecution.

***Response to Arguments***

In response to applicant's argument that there is no suggestion to combine the references, the examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988)and *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). In this case, all references are directed to the same scientific area and are published prior to the effective filing date of the instant application. Thus, this knowledge is generally available to one of ordinary skill in the art.

Applicants argue that GB 2 214 518 teaches the use of multiple samples. This does not appear to be correct as the same sample of sperm is used to measure the fluorescence of a PI stain for both living and all sperm in the sample and the % of live and dead sperm may be calculated from these two measurements after correction for background fluorescence. Thus, the same sample of sperm is used for both determinations. It is considered to be a substantially simultaneous determination because of the lack of precision in the definition of "substantially" simultaneous.

Applicants state that in the method of US 4,559,309, the sample is stained with Rhodamine 123 and ethidium bromide. Rhodamine 123 stains the mitochondria in the tail and middle part of the sperm cell, while EB stains the head of the sperm cell. Since a semen sample may comprise up to 30% loose tails or heads, the total concentration of sperm cannot be very accurately determined. While this may be true, the claimed method does not have any accuracy limitations, but merely requires that the same sample or diluted sample of sperm be used to determine live and dead sperm at substantially the same time. US 4,559,309 fulfills the limitations of the method AS CLAIMED because it subjects the sperm to selective staining and determines the sperm number or concentration of sperm and the % of live sperm or sperm viability as well as sperm motility in a single FCM measurement (col. 8, ls. 11-17).

Applicants argue that the method of JP 8-332098 teaches the labeling where the concentration of sperm tends to be overestimated. Please note that no limitations are found in the claim with respect to accuracy. Even if there were such limitations in the claim, no objective evidence has been presented to demonstrate that the cited prior art does not achieve such accuracy.

Applicants argue that LIVE/DEAD Sperm Viability Kit or Garner et al. do not disclose a method to determine the concentration and proportion of live sperm cells substantially simultaneously by any method. However, on page 2 or LIVE/DEAD, a sample of sperm is incubated with both PI and SYBR 14 (steps 3 and 5). The live cells fluoresce green while damaged or dead fluoresce red

(page 1). The sperm are analyzed using cytometry or fluorescence microscopy. Cytometry is the counting of cells and the Abstract and Summary of Garner et al. state that the cells are quantified. Since the volume is known and the number of cells is known, the concentration or sperm count is also known.

Applicants argue that EP 586 183 and WO 93/16385 were developed for blood samples and that it is improper to combine them with methods for assessing the viability of sperm. Please note that EP 486 183 teaches in the Abstract, Summary of the Invention that the addition of an internal standard comprising a small fluorescent bead is useful in flow cytometry in cell counting methods. This is a generic teaching. Further, in the Detailed Description, it is stated that the sample (to be quantitated) may be derived from any tissue source or cell line. This is also a generic teaching. Thus, the addition of the internal standard, which is a fluorescent bead, to any cell sample which is being analyzed by flow cytometry is generically taught by EP 156 183 to permit direct determination of cell concentration.

WO 93/16385 teaches the addition of fluorescent beads (particles) as an internal standard to cells for use in a flow cytometer and generically mentions the addition of such a particle to a cell specimen (p. 4, l. 8). The claims of this application are not limited to blood cells, but like the generic disclosure appreciate the universal application to any cell being analyzed by flow cytometry. Applicants further argue that there is no indication that sensitive live sperm cells would be able to survive the determination procedure (as disclosed by EP'183 or WO '385). However, there does not appear to be any negative statements in either EP'185, or WO'385 concerning the ability of sperm cells to withstand the addition of fluorescent beads and there is no evidence in the case which demonstrates that those of skill in the art doubted that sperm cells could survive the addition of fluorescent beads. Thus, the argument is unpersuasive.

Applicant appears to argue unexpected results over "traditional methods" but does not carefully compare the cited prior art results with applicants'

results. Careful attention to the exemplified method might advance prosecution.

Applicants argue that no motivation exists in the prior art to combine the teachings of the primary reference(s), Garner et al. or LIVE/DEAD or US 4,559,309 or GB2 214 518 with WO 93/16385 or EP 586 183. Motivation has been explained above and further expanded upon here. The generic teaching of WO 93/16385 or EP 586 183 is that the addition of a fluorescent bead to a cell sample to be analyzed by flow cytometry permits the simultaneous determination of concentration of cells as well as any other parameters being analyzed (abstract WO 93/16385). Likewise, EP '183 suggests the addition of a fluorescent bead to any cell sample being analyzed by flow cytometry to permit absolute cell concentration to be determined in one pass through.

The Examiner understands that conventional flow cytometry does not permit a determination of absolute concentration of cells and percentage viable to be made simultaneously using flow cytometry alone. However the method as claimed uses the term "substantially simultaneously" which is indefinite and permits various interpretations. The claimed method also does not state of what the total concentration of sperm cells is in. For example, is it the total concentration in the original semen ejaculate or is it the concentration in the liquefied sample or is it the total concentration in the subsample (diluted sample which is run through the flow cell). Attention to claim language might remove some of the rejections and further prosecution

*Conclusion*

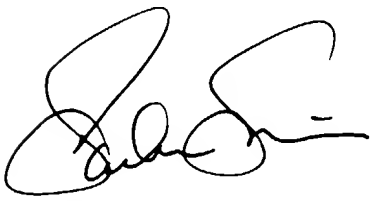
Applicants' amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, THIS ACTION IS MADE FINAL. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is

filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1651. The supervisor for 1651 is M. Wityshyn, (703) 308-4743. The normal work schedule for Examiner Saucier is 8:30AM to 5:00PM Monday and Tuesday and 8:30 AM to noon on Wednesday.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sandra Saucier whose telephone number is (703) 308-1084. Status inquiries must be directed to the Customer Service Desk at (703) 308-0197 or (703)-308-0198. The number of the Fax Center for the faxing of official papers is (703) 872-9306.



Sandra Saucier
Primary Examiner
Art Unit 1651
October 28, 2003